be differentially controlled from our original nanowalker via changes in buffer conditions that favor one spider over the other and vice versa. This advance will allow us to devise a controlled "spider race" towards a common goal post; depending on the predetermined winner, a payload will either be released from the goal or not, behaving as an XOR gate. By fluorophore labeling the different components, we aim to characterize this spider race at the single molecule level using super-accuracy total internal reflection fluorescence microscopy (TIRFM).

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839-Pos Board B639
Single Molecule Myosin V Dynamics Using High Time Resolution Polarized TIRF
John F. Beausang, Philip C. Nelson, Yale E. Goldman.
Translocation of the molecular motor myosin along its actin filament track was studied using polarized total internal reflection (polTIRF) microscopy, a technique that determines the 3D orientation and dynamic wobble of single fluorophores. The maximum time resolution of the experimental setup was increased 50-fold by cycling through 8 different input laser paths and linear polarizations in approximately 1 ms. The arrival time and polarization state of each photon was recorded using a modified time-correlated single photon counting module. A new analysis, the Multiple Channel Change Point algorithm, was developed to detect changes in molecular orientation and wobble using the raw time-stamped data with no user-defined bins or thresholds. The analysis objectively identified changes in orientation and wobble of each fluorophore with a time resolution limited only by the photon counting rate. The polarized fluorescence from single rhodamine probes bifunctionally attached to one of the calmodulins on the myosin V lever arm was recorded as the motor translocated along a fixed actin filament in the presence of ATP. At the low (10 uM) ATP concentrations used here, the time intervals between sudden polarization changes averaged 120 ms and corresponded to stable orientations of the lever arm after each step. Occasionally, 10–80 ms substeps with increased probe wobble were detected between the longer duration dwells, possibly corresponding to the putative diffusive search of the detached head for its next actin binding site. Periods of large wobble were also detected at the end of many runs and when molecules were attached to actin in the absence of ATP. In both situations motors are probably bound to actin by only one head. This work was supported by NSF grants DMR-0832802, EF-0928048, IGERT DGE02-21664 and NIH grant GM-086352.

840-Pos Board B640
Hyperspectral Line Scanning Microscopy for High-Speed Multicolor Quantum Dot Tracking
Michael Malik, Patrick J. Cutler, Sheng Liu, Jason Byars, Diane Lidke, Keith Lidke.
One of the fundamental goals in observing protein-protein interactions on the cell membrane is achieving nanometer scale spatial resolution along with temporal resolution sufficient to study live cell behavior. Traditional fluorescence microscopy methods have been unsuccessful in studying these interactions due to the diffraction limit with visible light. Single particle tracking techniques using quantum dots have provided single particle localizations to well below the diffraction limit, however, clustering of multiple particles limits the unique identification and thus tracking of individual particles throughout the (possibly dynamic) clustering process. This problem can be solved by tracking multiple quantum dot colors using a high-speed hyperspectral microscope.
We have developed a high-speed hyperspectral microscope based on a line scanning design that is capable of up to 30 frames/sec with 128 spectral channels per pixel (ranging from 500-800 nm) with a sample area of ~30 υm^2. We describe the details of the microscope optical design details and layout, and show the results of excitation and emission path characterization. We image RBL-2H3 cells with IgE that has been conjugated with various colors of QDs (to give a near 1:1 QD:IgE ratio) and image at 30 frames per second. By imaging in both the presence and absence of crosslinking DNP-BSA, we demonstrate the ability to identify dynamic interactions and/or oligomers at spatial scales below the diffraction limit.

841-Pos Board B641
In-Vivo, Single-Molecule Characterization of the MinCDE System's Localization and Dynamics
Jackson A. Buss.
In bacteria cell division, the accurate placement of the septal ring at midcell is of inherent importance. One important system in E. coli that performs this task is the MinCDE system, which prevents peripheral division complex formation through the pole-to-pole oscillation of MinC, a known FtsZ antagonizer. It was proposed that this oscillatory redistribution is driven by the molecular interactions intrinsic to MinD and MinE, namely cooperative membrane-bound self-assembly and regulation of membrane association-dissociation via ATP binding and hydrolysis. However, the molecular details of this proposal are difficult to verify in vivo using conventional ensemble-based methods due to the highly dynamic nature of the system. In this work we employ single-molecule techniques in live E. coli cells to identify structural details of the previously reported cytoskeleton-like, helical framework of the Min proteins with 30-nm spatial resolution, and their membrane-associated dynamics with millisecond time resolution. We provide evidence elucidating molecular mechanisms responsible for the periodic, self-organized behavior of the system, shedding light on the mechanism employed to govern proper placement of the septum.

842-Pos Board B642
Measuring Hopping Energies of Individual Biomolecules
Jean-Baptiste Masson, Silvan Türkcan, Marianne Renner, Maxime Dahan, Antigoni Alexandrou.
Hopping is associated to escaping events of systems that were previously confined or localized. In biology, hopping happens when membrane proteins locally confined "jump" to another confinement domain, when proteins sliding on DNA leave the double strand to diffuse in the local environment or when some neurotransmitters move near synapses.
We recently implemented a novel inference approach that exploits the full information stored in a biomolecule trajectory to extract force [1] and diffusivity maps [2] from non-equilibrium tagged biomolecule motion. We showed that, when the motion is confined, only a few hundred points were necessary to extract precise and accurate maps.
We here introduce a new inference scheme, still based on biomolecular motion, able to extract hopping energy of escaping biomolecules. A potential energy, developed in a polynomial basis, is inferred from the motion of the biomolecule and the average hopping energy (as well as its standard deviation) is extracted from the Monte Carlo sampling of the Posterior distribution of the potentials. Inference performed on numerical trajectories shows good agreement between the extracted and the input values even when there is a unique hopping event. We apply this method to various membrane proteins exhibiting both 1D and 2D motion.
This method allows quantitative measuring of biochemical data from individual biomolecules in vivo.
[1] J.-B. Masson et al, PRL 102, 048103 (2009).
[2] G. Voisinne et al, Biophys. J. 98(4), pp 596–605 (2010).

843-Pos Board B643
Accommodating Drift in Hidden Markov Analysis of Single-Molecule Data
George A. Emanuel, Tatiana Kuriabova, Amanda E. Carpenter, M.D. Betterton, Thomas T. Perkins.
Single-molecule techniques are increasingly used to measure state transitions in biomolecules. These data are typically a noisy time series with discrete transitions that reflect underlying distinct states; examples include the opening and closing of DNA hairpins, and the folding and unfolding of proteins. Hidden Markov models (HMMs) have been successfully used to infer transitions between the underlying states from noisy data. However, HMMs are typically applied to ratiometric data (e.g., FRET studies of RNA folding) and do not perform well on data that include drift. Yet, drift is common in real-space records from optical traps and atomic force microscopes. We developed a HMM that accommodates experimental drift using low-order Fourier modes. We use simulated traces to demonstrate the improved performance of our method and illustrate its use on measurements of single TATA-box binding proteins bending and unbending DNA.

844-Pos Board B644
Extending Fragment Based Free Energy Calculations with Library Based Monte Carlo Simulation: Annealing in Interaction Space
Steven Lettieri, Artem B. Mamonov, Daniel M. Zuckerman.
Pre-calculated libraries of molecular fragment configurations have previously been used as a basis for both equilibrium sampling (via "library-based Monte Carlo") and for obtaining absolute free energies using a polymer-growth formalism. Here, we combine the two approaches to extend the size of systems for which free energies can be calculated. We study a series of all-atom poly-alanine systems in a simple dielectric "solvent" and find that precise free energies can be obtained rapidly. For instance, for 12 residues, less than an hour of single-processor is required. The combined approach is formally equivalent to the "annealed importance sampling" algorithm; instead of annealing by decreasing temperature, however, interactions among fragments are gradually added as the molecule is "grown." We discuss implications for future binding affinity calculations in which a ligand is grown into a binding site.